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(54) Title: HUMAN GALANIN, cDNA CLONES ENCODING HUMAN GALANIN AND A METHOD OF PRODUCING HUMAN GALANIN

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|-----------|------|------|------|------|-----------------|------|------|------|------|------|------|------|------|
| human GAL | GLY- | TRP- | THR- | LEU- | ASN- | SER- | ALA- | GLY- | TYR- | LEU- | LEU- | GLY- | PRO |
| bovine | | | | | | | | | | | | | |
| porcine | | | | | | | | | | | | | |
| rat | | | | | | | | | | | | | |
| | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
| human | HIS- | ALA- | VAL- | GLY- | ASN- | HIS- | ARG- | SER- | PIR- | SER- | ASP- | LYS | ASN- |
| bovine | | | | LEU- | ASP- | SER- | | | | GLN- | | | HIS- |
| porcine | | | | ILE- | ASP- | ASN- | | | | HIS- | | | TYR- |
| rat | | | | ILE- | ASP- | ASN- | | | | SER- | | | HIS- |
| | 27 | 28 | 29 | 30 | | | | | | | | | |
| human GAL | GLY- | LEU- | THR- | SER | | | | | | | | | |
| bovine | | | | ALA- | NH ₂ | | | | | | | | |
| porcine | | | | ALA- | NH ₂ | | | | | | | | |
| rat | | | | THR- | NH ₂ | | | | | | | | |

(57) Abstract

The present invention provides a peptide having the amino acid sequence of human galanin. The amino acid sequence of this peptide is: GWTLSAGYLLGPHAVGNHRSFSDKNGLTS. The present invention further provides DNA clones encoding the peptide and to therapeutic uses of the peptide.

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Human Galanin, cDNA Clones Encoding Human Galanin and a
Method of Producing Human Galanin

Field of the Invention

The present invention relates to a peptide having the
5 amino acid sequence of human galanin as deduced from the
nucleotide sequence of human preprogalanin cDNA. The
present invention further relates to cDNA clones encoding
the peptide. In addition, the present invention
encompasses therapeutic uses of the peptide, and the use
10 of the peptide in designing galanin antagonists and
agonists.

Background of the Invention

Galanin is a putative neuropeptide which was first
isolated from porcine small intestine in 1983(1). Porcine
15 galanin is a peptide of 29 amino acid residues which was
named for its N-terminal glycine and amidated C-terminal
alanine residues(1). The cDNAs encoding galanin have been
cloned from three species, rat (2), porcine (3) and bovine
(4), revealing that galanin is a proteolytic product of a
20 larger precursor protein known as preprogalanin(2).
Galanin shows 90% homology between the species but little
similarity to other known peptides(1). Antibodies raised
to porcine galanin have allowed the mapping of
galanin-like-immunoreactivity (GAL-LI) to discrete regions
25 of the Central Nervous System (CNS) and throughout the
Peripheral Nervous System (PNS) of several other species
including man.

Immunohistochemical mapping of GAL-LI in the CNS has
been performed most intensively in the rat where the
30 highest concentrations have been found in the median
eminence and hypothalamus(5). These results are
consistent with more recent in situ hybridisation studies
where the localisation of preprogalanin in the rat brain
tentatively suggests the involvement of galanin in the
35 feeding regulation of several factors ranging from water

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balance behaviour to blood pressure control(6).
Similarly, radioimmunoassay of galanin in the baboon brain
showed high GAL-LI in the hypothalamus and median
eminence, and also GAL-LI in association with limbic
5 structures such as the amygdala(7). Immunohistochemistry
and in situ studies of preprogalanin mRNA during
development of the rat has shown tissue specific sex
differences in galanin concentration, notably in the
anterior pituitary(5) where its expression is oestrogen
10 dependent(9). The overall distribution of GAL-LI and its
colocalisation in discrete neuronal cells with
catecholamines, serotonin, GABA, acetylcholine and various
other peptides (10) strongly suggest a modulatory role for
galanin. A noteworthy example is the coexistence of
15 galanin with acetylcholine in nerve fibers projecting from
the basal forebrain to the hippocampus, in the rat (11)
and baboon (7) which has led to speculation that galanin
may play a role in Alzheimers disease. There is, however,
conflicting evidence concerning the expression of galanin
20 in this region of the human brain. Although the
physiological role of galanin in the CNS has not yet been
established its pharmacology suggests a role in
neuroendocrine regulation. Injection of galanin into the
third ventricle of rats causes increased growth
25 hormone(13) and injection into the paraventricular nucleus
(PVP) enhances food intake(14).

In the PNS, distribution of GAL-LI suggests that
galanin is widespread. Galanin distribution and its
pharmacology, which is diverse and often species specific,
30 both suggest a range of physiological actions for
galanin. However, some confusion may have arisen as to
its pharmacological role through the use of porcine
galanin in experiments involving other species. In
numerous mammalian species the highest concentrations of
35 GAL-LI are found in the intestine(1), pancreas(15),

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adrenal glands (3), and respiratory(16) and genitourinary tracts (17). Galanin action on the pancreas and its possible role in diabetes is controversial; it has been established that porcine galanin infusion in dogs(15), and
5 rat and porcine galanin perfusion through the isolated rat pancreas(18), decrease plasma insulin levels. However there are conflicting results concerning porcine galanin action on the pig pancreas(19). In the dog galanin also decreases somatostatin while increasing glucagon but this
10 may not be the case in other species(15). Intravenous porcine galanin causes growth hormone secretion in a variety of species including man. However, intravenous porcine galanin infusion in man at a concentration sufficiently high to elicit an increase in growth hormone
15 levels, does not cause the expected inhibition of insulin (20). The apparent discrepancy may be due to the difference in amino acid sequence of human verses porcine galanin, or it may be simply a reflection of the species specific effects of galanin. Visualisation of GAL-LI in
20 neurons innervating the islets of several species (15) added to a proposal to explain the galanin induced inhibition of insulin secretion in rat B-cell lines (21) support a neuromodulatory role for galanin on endocrine pancreatic action. Other pharmacological effects of
25 galanin in the PNS include the species specific stimulatory or inhibitory action of galanin on the smooth muscle activity of several mammalian species (22).

Galanin receptors have been identified in a hamster insulin-secreting B-cell tumor (23), rat (24) and monkey
30 brain (25), and smooth muscle membranes (22). The distribution of galanin binding correlates with that of GAL-LI and therefore supports the role of galanin in neurotransmission. It is not clear whether there are subtypes of the galanin receptor, nor which region of the
35 peptide is responsible for binding to its receptor.

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Studies on the biological effect of tryptic fragments of galanin on smooth muscle preparations (22), in addition to auto-radiographic binding studies on Rin 5mf pancreatic B cell-lines (26) and on intestinal membrane preparations
5 (27), present conflicting results.

The molecular biology of the galanin gene has not yet been examined in humans. Porcine preprogalanin is a 123 amino acid residue protein that comprises a signal sequence, galanin (29 amino acids) and a 59 amino acid
10 peptide known as galanin mRNA associated peptide (GMAP). The length and structure of rat porcine and bovine preprogalanin are similar. The 20% difference in galanin amino acid homology across the species is manifest over the C-terminal end of the peptide. The sequence in all
15 species identified to date suggests post translational cleavage of glycine extended galanin followed by amidation. GMAP is also well conserved across the species which has led to speculation that it is biologically active; it includes a region of 35 amino acids that shows
20 78% homology across the species and within this region a stretch of 17 residues that shows greater homology.

This invention discloses the isolation and characterisation of human preprogalanin from a neuroblastoma cell line cDNA library and from a pituitary
25 cDNA library (28). Oligonucleotides complementary to two conserved regions of pig and rat preprogalanin were used in a polymerase chain reaction (PCR) to specifically amplify the corresponding sequence from neuroblastoma and pituitary cDNA. The two amplification oligonucleotides
30 used (No. 1 and 2) correspond to amino acids 29-37 and 105-97 of rat and pig preprogalanin respectively, and flank a 230 basepair region encoding galanin and the N-terminus of GMAP (Fig. 1) (29). An additional oligonucleotide (No. 3) within this region was used to
35 probe for the correct PCR product (30). The amplified

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region from both sources of DNA was subcloned and then sequenced (31), revealing identical sequences. The region amplified from neuroblastoma cDNA was used as a probe to isolate clones encoding the complete preprogalanin cDNA
5 from this library (32). Later, the pituitary cDNA library was screened with the same probe, in order to ascertain that any amino acid differences apparent between human preprogalanin and other species, were not due to the erroneous translation of DNA in the neuroblastoma cultured
10 cell line.

The primary structure of human preprogalanin cDNA clones isolated from both libraries were identical but different to that of pig, cow and rat. In general, amino acid substitutions only occurred at positions noted for
15 variability amongst the other species (Fig. 2), thus confirming that galanin, and GMAP to a lesser extent, are both well conserved. However, several of these changes (e.g. 17, 23 and 26 in galanin) involve amino acids with very different physical and chemical properties suggesting
20 that such changes are important for the correct function of human galanin. Also important is that human galanin is rendered unique by the striking substitution of a serine for a glycine residue at the C-terminus of galanin, directly proceeding the lys-arg cleavage site in the
25 precursor protein. This implies that human galanin is not amidated at its C-terminus, in contrast to other species, where the glycine residue serves as an amide donor to the proceeding residue after proteolysis. Consequently, human galanin may have a variety of biological properties that
30 differ from porcine, rat, and bovine galanin.

Summary of the Invention

Accordingly, in a first aspect the present invention consists in a polypeptide having the following amino acid sequence:-

35 GWTLSAGYLLGPHAVGNHRSFSDKNGLTS

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or a functional equivalent thereof or a fragment thereof.

In a preferred embodiment of the present invention the polypeptide fragment has the amino acid sequence:-

GWTLNSAGYLLGPHAVNHRFSKNGLT

5 As used herein in relation to polypeptide sequences the term "functional equivalent" is intended to cover minor variations in the amino acid sequence which do not deleteriously affect the biological activity of the polypeptide.. It will be recognised by those skilled in
10 the art that a number of modifications may be made to the peptide of the present invention without deleteriously affecting the biological activity of the peptide. This may be achieved by various changes, such as insertions, deletions and substitutions, either conservative or
15 non-conservative in the peptide sequence where such changes do not substantially decrease the biological activity of the peptide. By conservative substitutions the intended combinations are:-

G, A; V, I, L, M; D, E; N, Q; S, T; K, R, H;
20 and F, Y, W.

It may also be possible to add various groups to the peptide of the present invention to confer advantages such as increased potency or extended half-life in vivo without substantially decreasing the biological activity of the
25 peptide. Peptides designed to perform these functions are described as galanin agonists. These additions and changes include the introduction of D-amino acid residues and the formation of cyclic analogues.

In a second aspect the present invention consists in
30 a cDNA molecule encoding the peptide of the present invention, the cDNA molecule having a sequence substantially as shown in Figure 1 from nucleotide 97 to 186 or a functionally equivalent sequence.

In a third aspect the present invention consists in a
35 DNA molecule encoding human preprogalanin and GMAP, the

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DNA molecule having a sequence substantially as shown in Figure 1 or a functionally equivalent sequence.

As used herein in relation to DNA sequences the term "functionally equivalent sequence" is intended to cover
5 minor variations in the DNA sequence which, due to degeneracy in the DNA code, do not result in the sequence encoding different polypeptides.

Further, this term is intended to cover alterations in the DNA code which lead to changes in the encoded
10 peptide, but in which such changes do not effect the biological activity of the peptide.

A different half life for human galanin in vivo can be expected, in addition to differences in binding affinity for specific receptors and thus potency between
15 different species.

Of particular interest will be the effect of human galanin on insulin inhibition. Infusion of both porcine and rat galanin through an isolated rat pancreas showed that both types of galanin inhibited insulin and
20 somatostatin release, although porcine galanin was less potent than rat galanin (18). In addition, rat galanin enhanced glucagon secretion whereas porcine galanin was ineffectual. The difference in activity of pig and rat galanin has been ascribed to the 4 amino acid differences
25 that exist between them at their C-terminus. Similarly, the 5 amino acids that differ between human and porcine galanin, coupled with the difference due to amidation, may be responsible for the lack of expected insulin inhibition observed when porcine galanin was infused into
30 human subjects (20). Controversy concerning galanin action on the pancreas, and other examples of the species specific effect of galanin, such as the effect of galanin on the GI tract, indicate that it is preferable to use galanin homologous to the species under investigation.

35 In a fourth aspect the present invention consists in

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a method of producing human galanin comprising culturing a cell transformed with the cDNA molecule of the second or third aspect of the present invention under conditions which allow expression of the DNA sequence and recovering
5 the human galanin.

While it is possible to form the polypeptide of the present invention by biological means involving recombinant techniques in prokaryotic or eucaryotic cells the polypeptides may also be formed by chemical
10 synthesis. The decision as to which route of synthesis is used will depend primarily on the length of peptide to be synthesised.

From preliminary results it is clear that the polypeptide of the present invention has therapeutic
15 application in modulating pancreatic activity, as a stimulator of growth hormone and as an attenuator of cardiac vagal function.

Accordingly, in further aspects the present invention consists in a method of modulating pancreatic activity,
20 stimulating the production of growth hormone or attenuating cardiac vagal function in a human comprising administering the peptide of the present invention to the human.

The present invention also consists in the use of the
25 polypeptide of the present invention in the preparation of a medicament for modulating pancreatic activity, stimulating the production of growth hormone or the attenuation of cardiac vagal function.

It has been demonstrated that galanin antagonists can
30 be developed by chemical synthesis of chimeric galanin-like peptides. The N-terminal galanin fragment (amino acids 1-13), which binds the galanin receptor, can be coupled to a peptide of an α -helical structure that stabilises the N-terminal portion but has no innate
35 biological action. The resulting chimera is described as

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a galanin antagonist, since it will bind but not activate the galanin receptor, thus inhibiting the action of endogenous galanin. The ability of such a chimeric peptide to function as galanin antagonist can be assessed
5 by measuring its binding efficiency to galanin receptors expressed in RIN m5F cells and the ability to reverse the inhibited insulin response to galanin. A galanin antagonist will displace 125I-galanin binding from RIN m5F cells in a dose dependant manner and reverse the inhibited
10 glucose-induced insulin response to galanin. The antagonist functions as a competitor to galanin but has no effect itself on glucose-induced insulin secretion.

Using the polypeptide of the present invention it will be possible to screen peptides other compounds for
15 galanin agonist and antagonist activity. This would be doen by receptors impressed in RIN m5F cells. The compounds which showed competitive binding would then be assesses for biological activity.

In another aspect the present invention consists in a
20 method of screening compounds for galanin agonist or antagonist activity comprising assessing the ability of the compound to compete with the peptide of the present invention for binding to cell receptors and assessing the biological of the compounds which competitively bind.

25 The present invention also relates to galanin antagonists obtained by this screening method.

In order that the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples and
30 accompanying Figures in which:-

Fig. 1 shows the nucleotide sequence of preprogalanin, the amino acid sequence of human galanin and GMAP;

Fig. 2 provides a comparison of the amino acid sequence of human galanin with that of bovine, porcine,
35 and rat;

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Fig. 3 shows the effect of administration of human galanin on levels of blood glucose (BG; (a) 350 μ g and (b) 250 μ g) and serum insulin levels (c 250 μ g) in the conscious rat. The arrow indicates the time point at which the galanin was

5 administered;

Fig. 4 shows the experimental protocol for infusion of human galanin into humans;

Fig. 5 shows the effect of administration of human galanin on pulse rate in a human subject.

10 Fig. 6 shows the effect of administration of human galanin on plasma glucose and insulin levels in a human subject (—●— saline; —○— 1×10^{-9} M human galanin —□— $3-4 \times 10^{-9}$ M human galanin; Arrows 1 and 3 show commencement and stoppage of galanin infusion respectively

15 and arrow 2 shows when glucose administered).

Fig. 7 shows the effect of administration of human galanin or growth hormone levels in a human subject (—□— saline; —●— 1×10^{-9} M human galanin; —■— $3-4 \times 10^{-9}$ M human galanin).

20 EXAMPLES

MATERIALS AND METHODS

cdNA Libraries:

Two cdNA libraries were used for library screening and also as a source of template DNA for a polymerase chain reaction (PCR). The neuroblastoma cdNA library (cat No. HL1007, Clontech Laboratories Inc., USA) contained 1.05×10^6 independent clones inserted into a λ gt10 vector at its EcoRI cloning site. The pituitary cdNA library was obtained from Dr P. Seeburg (centre for Molecular Biology, University of Heidelberg, FRG) and was also carried in λ gt 10 and cloned in the EcoRI site.

Oligonucleotide Synthesis:

With the exception of oligonucleotides directed to the EcoRI cloning site of λ gt10 (Promega, VIC., Australia), all oligonucleotides were prepared on a DNA

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synthesiser (Applied Bio-systems, DNA synthesiser Model 380B, Burwood, Australia).

oligonucleotide sequence:

1. GAATTCAAGGA(A/G)AAGAGAGGCTGGAC(T/C)CTGAA (EcoRI site
5 incorporated)
2. CCATAAGCTTGC(G/C)CC(G/C)GC(G/A/T/C)TCTTT(A/G)AG
(A/G)TGCA(G/A)GAA (HindIII site incorporated).
3. CCATAAGCTTAATGA(C/T)CTGTGG(C/T)TGTC(A/G)A(T/G)(C/G)
GCATG (HindIII site incorporated)

10 Polymerase chain reaction (PCR):

In order to prepare template cDNA, a plate lysate method was used to propagate phage (T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning: A Lab Manual, 2Ed., Cold Spring Harbour Press, USA, 2.65 (1989)). The
15 libraries were plated at 20,000 plaques per 150mm diameter plate and extracted into storage medium (SM=0.1M NaCl/0.008M MgSO₄·7H₂O/0.05M Tris.HCl/0.02% gelatin) which gave a preparation with a titre of 1x 10⁹ phage per ml. The phage stock (2ml) was extracted with
20 phenol/CHCl₃ and then precipitated with ethanol.

The PCR was used to amplify a 230 base pair region of preprogalanin from the neuroblastoma cDNA library (oligos 1 and 2) and also to amplify the cDNA library clones (λgt10 oligos) that were later isolated by screening.
25 In both reactions, a Hybaid intelligent heating block (Model 1HB 2024, Hybaid, Middx., UK) was used with the following temperature parameters: hold at 95 °C(5'), then 25 cycles of 92 °C(1'), 42 °C(1') and 72 °C(1'). Each reaction contained KCl (50mM), gelatin (100ug/ml), MgCl₂
30 (1.5mM), Tris-HCl (pH=8, 10mM), DNA (10ng-100ug), dNTP (200uM), Tth polymerase (0.25U, Toyobo, Japan) and oligonucleotides (500uM).

PCR products were separated on a 3% Nuseive gel (FMC Bioproducts, ME, USA) (products less than 500 basepairs) or
35 1% agarose (products greater 700 basepairs). The identity

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of PCR product bands was established by Southern blotting (E Southern, J. Mol. Biol, 98, 503 (1975)) the DNA onto a nylon membrane (Zeta probe, Bio-Rad Laboratories Inc., CA, USA) using 0.4M NaOH as the transfer buffer followed by hybridisation with oligo #3. Pre-hybridisation was performed at 42°C in a solution of 5 x SSPE (1X SSPE=0.18M NaCl.10mM NaH₂PO₄/1mM NaEDTA pH=7), 0.5% sodium dodecyl sulphate (SDS) and 5 x Denhardt's solution (1 x Denhardt's solution = 0.02% Ficoll-400/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone-40) and 100µg/ml heat denatured salmon sperm DNA. Hybridisation was performed in the same solution with the addition of probe for 6-12 hours at 42°C. The oligo was labelled with γ -³²P ATP Amersham, International Plc, UK) using T4 polynucleotide kinase (BRL, MD, USA). Blots were washed at 37°C in 1 x SSC (1 x SSC=0.151M NaCl/0.1675 M tri-sodium citrate)/ 0.1% SDS prior to exposure to X-ray film (Kodak Eastman, NY, USA) at -70°C with an intensifying screen for 12 hours.

20 Subcloning and sequencing:

PCR products were separated on a gel as described above. The appropriate band was excised and purified by using gene clean (Bio 101, CA, USA), before restriction digestion. The PCR product generated by preprogalanin oligos 1 and 2 was digested with HindIII and EcoRI, whereas the PCR product generated with the γ t10 oligos was digested with EcoRI, before ligation into M13mp19. The M13mp19 subclones were used to transform JM101 competent cells (Maniatis, 1.82-1.84) and subsequently single stranded DNA was prepared using standard methods (Maniatis, 4.29-4.30) for sequencing (kit No. Q5800, Promega). Sequencing difficulties due to secondary structure were overcome with the use of Taq DNA polymerase (kit No. Q 5540, Promega), as the sequencing enzyme at a reaction temperature of 70 °C.

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cdNA Library Screening:

The cdNA libraries described above were screened with the 230 base pair PCR product encoding the sequence of neuroblastoma preprogalanin. The probe was excised from a gel and purified using gene clean before labelling (25ng) with α^{32} dCTP in a random priming reaction (kit No. 8187SA, BRL). Approximately 6×10^5 plaques were plates on 2YT plates (A3, Maniatis) lifted onto High Bond N nylon filters (Amersham) and fixed according to the manufacturer's recommendations. Pre-hybridisation and hybridisation were performed at 65°C as described above. Filters were washed to a stringency of 0.1% SDS / 0.1% SSC at 65°C and exposed overnight to X-ray film with intensifying screens.

15 Blood Glucose and Secretion of Gluco-Regulatory Hormones
The Administration of Human Galanin to Conscious Rats

Rats were maintained on established diets and cannulated under anaesthesia. The rats were then allowed to recover and infused with glucose. Ten minutes later a bolus dose of human galanin was administered and blood samples were taken over the next three hours. Following the sampling procedures the animals were sacrificed.

An elevation of blood glucose levels was observed in response to bolus administration of $350 \mu\text{g}$ (110 nmol; Fig. 2a) and $250 \mu\text{g}$ (80 nmol; Fig 2b) human galanin. the elevation of blood glucose levels in response to the administration of $250 \mu\text{g}$ (80 nmol in Fig. 2c) human galanin correlates with a drop in levels of circulating insulin.

30 Growth Hormone Secretion

Infusion of Human Galanin into Humans

The experimental protocol for the infusion of human galanin into humans to achieve maximal circulating levels of galanin of approximately $3-4 \times 10^{-9}\text{M}$ is shown in Figure 4.

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The effect of human galanin on blood glucose and secretion of glucoregulatory hormones in humans

Preliminary data for one subject indicate that the administration of human galanin according to the protocol described in Fig. 4, to achieve maximal circulating levels of 1×10^{-9} M and 4×10^{-9} M human galanin, resulted in a detectable suppression of insulin secretion (Fig. 6; Y-Axis units : mIU/L). This was associated with an elevation of plasma glucose relative to the control situation (Fig. 6; Y-Axis units : mM).

The effect of human galanin on growth hormone secretion

The administration of human galanin to human volunteers according to the protocol described in Fig. 4 resulted in an elevation of growth hormone levels at circulating levels of both 1×10^{-9} M and $3-4 \times 10^{-9}$ M human galanin in the two subjects studied to date. The effect of human galanin in one of these two subjects at the two dosage rates employed is shown in Fig. 7.

Cardiovascular Effects

The effect of human galanin on blood pressure and vagal nerve function in the anaesthetised cat

The intravenous injection of human galanin into anaesthetised cats resulted in an attenuation of cardiac vagal slowing of heart rate.

Cardiovascular effect of human galanin in humans

The infusion of human galanin into humans as detailed in Fig. 4 resulted in an increase in pulse rate (see Fig. 5), consistent with an effect of human galanin on attenuation of vagal function in the human.

From studies conducted to date it is believed that human galanin will have a number of therapeutic uses including:-

1. Inhibition of gastrointestinal activity, e.g. as an antidiarrhea agent.
2. Inhibition of insulin secretion e.g. modulate

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activity of endocrine pancreas in pancreatic disorders.

3. As a potent stimulator of growth hormone which acts independently of growth hormone releasing hormone.
- 5 4. As an attenuator of cardiac vagal function.
5. As yet not well characterised effects in the nervous system e.g. its depletion in Alzeimers disease, effects on appetite, prolactin release etc.

Experiments conducted using rat models support the
10 first three uses, while studies in humans set out above with human galanin demonstrate the ability of human galanin to modulate insulin secretion, growth hormone secretion and cardiac vagal functions.

Prior to the present invention the possible
15 therapeutic uses of galanin were largely restricted due to the species specific pharmacological action of galanin i.e. exogenous human galanin when administered to humans would have a different pharmacological effect than commercially available pig and rat galanin. Prior to the
20 present invention the reasons for this species specificity was not understood. It is now believed that the species specificity is due, at least in part, to the several amino acid striking differences between human galanin and that of other species and of particular note is not extended by
25 glycine at its C-terminal, and that in its place is a serine residue which excludes the possibility of amidation after post-translational cleavage in vivo.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made
30 to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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CLAIMS:-

1. A polypeptide having the following amino acid sequence:-
GWTLNSAGYLLGPHAVGNHRSFSDKNGLTS
- 5 or a functional equivalent thereof or a fragment thereof.
2. A polypeptide as claimed in claim 1 in which the polypeptide fragment has the following amino acid sequence:-
GWTLNSAGYLLGPHAVGNHRSFSDKNGLTS
- 10 3. A cDNA molecule encoding the peptide as claimed in claim 1 or 2, the cDNA molecule having a sequence substantially as shown in Figure 1 from nucleotide 97 to 186 or from nucleotide 97 to 145 or a functionally equivalent sequence.
- 15 4. A DNA molecule encoding human preprogalanin and galanin mRNA associated peptide, the DNA molecule having a sequence substantially as shown in Figure 1 or a functionally equivalent sequence.
5. A method of producing human galanin comprising a cell
20 transformed with the DNA molecule of claim 3 or 4 under conditions which allow the expressions DNA sequence and recovering the human galanin.
6. A method as claimed in claim 5 in which the cell is a bacteria.
- 25 7. A method of modulating pancreatic activity in a human subject comprising administering to the subject a therapeutically effective amount of the peptide as claimed in claim 1 or claim 2.
8. A method of stimulating the production of growth
30 hormone in a human subject comprising administering to the subject a therapeutically effective amount of the peptide as claimed in claim 1 or claim 2.
9. A method of attenuating cardiac vagal function in a human subject comprising administering to the subject a
35 therapeutically effective amount of the peptide of claim 1

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or claim 2.

10. A method of screening compounds for galanin agonist or antagonist activity comprising assessing the ability of the compound to compete with the peptide as claimed in claim 1 or claim 2 for binding to cell receptors and assessing the biological of the compounds which competitively bind.

Fig 1 1/7

Base Pair Number -20 -10
 5' * * * * * AGCGCAGCTCAAG

10 20 30 40 50 60
 * * * * *
 ATGGCCCCGAGGCAGCGCCCTCCTGCTCGCCTCCCTCCTCGCCGCGGCCCTTTCTGCC
 MetAlaArgGlySerAlaLeuLeuLeuAlaSerLeuLeuLeuAlaAlaAlaLeuSerAla

70 80 90 100 110 120
 * * * * *
 TCTGCGGGGCTCTGGTCGCCGCGCAAGGAAAAACGAGGCTGGACCCTGAACAGCGCGGGC
 SerAlaGlyLeuTrpSerProAlaLysGluLysArgGlyTrpThrLeuAsnSerAlaGly

130 140 150 160 170 180
 * * * * *
 TACCTGCTGGGCCCACATGCCGTTGGCAACCACAGGTCATTACGCGACAAGAATGGCCTC
TyrLeuLeuGlyProHisAlaValGlyAsnHisArgSerPheSerAspLysAsnGlyLeu
 HUMAN GALANIN

190 200 210 220 230 240
 * * * * *
 ACCAGCAAGCGGGAGCTGCGGCCCCGAAGATGACATGAAACCAGGAAGCTTTGACAGGTCC
ThrSerLysArgGluLeuArgProGluAspAspMetLysProGlySerPheAspArgSer
 GALANIN mRNA ASSOCIATED PEPTIDE

250 260 270 280 290 300
 * * * * *
 ATACCTGAAAACAATATCATGCGCACAATCATTGAGTTTCTGTCTTTCTTGCATCTCAAA
IleProGluAsnAsnIleMetArgThrIleIleGluPheLeuSerPheLeuHisLeuLys

310 320 330 340 350 360
 * * * * *
 GAGGCCGGTGCCCTCGACCGCCTCCTGGATCTCCCCGCCGAGCCTCCTCAGAAGACATC
GluAlaGlyAlaLeuAspArgLeuLeuAspLeuProAlaAlaAlaSerSerGluAspIle

370 380 390 400 410 420
 * * * * *
 GAGCGGTCCTGAGAGCCTCCTGGGCATGTTTGTCTGTGTGCTGTAACCTGAAGTCAAACC
GluArgSerEnd

430 440 450 460 470 480
 * * * * *
 TTAAGATAATGGATAATCTTCGGCCAATTTATGCAGAGTCAGCCATTCTGTTCTCTTG

490 500 510 520 530 540
 * * * * *
 CCTTGATGTTGTGTTGTTATCATTTAAGATTTTTTTTTTTTTTGGTAATTATTTTGAGTG

550 560
 * *
 GCAAAATAAAGAATAGCAATTA (n)

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FIG 2

| | | | | | | | | | | | | | |
|-----------|------|------|------|-----------------|------|------|------|------|------|------|------|------|------|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | |
| human GAL | GLY- | TRP- | THR- | LEU- | ASN- | SER- | ALA- | GLY- | TYR- | LEU- | LEU- | GLY- | PRO |
| bovine | | | | | | | | | | | | | |
| porcine | | | | | | | | | | | | | |
| rat | | | | | | | | | | | | | |
| 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | |
| human | HIS- | ALA- | VAL- | GLY- | ASN- | HIS- | ARG- | SER- | PHE- | SER- | ASP- | LYS | ASN- |
| bovine | | | LEU- | ASP- | SER- | | | | GLN- | | | | HIS- |
| porcine | | | ILE- | ASP- | ASN- | | | | HIS- | | | | TYR- |
| rat | | | ILE- | ASP- | ASN- | | | | SER- | | | | HIS- |
| 27 | 28 | 29 | 30 | | | | | | | | | | |
| human GAL | GLY- | LEU- | THR- | SER | | | | | | | | | |
| bovine | | | ALA- | NH ₂ | | | | | | | | | |
| porcine | | | ALA- | NH ₂ | | | | | | | | | |
| rat | | | THR- | NH ₂ | | | | | | | | | |

SUBSTITUTE SHEET

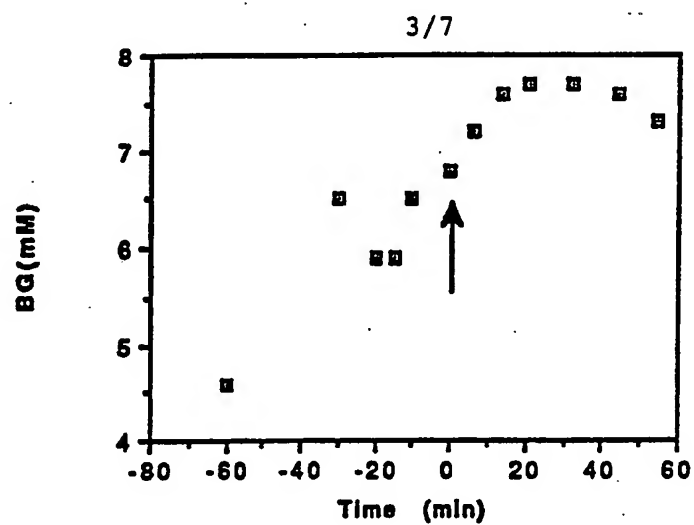


FIG 3A

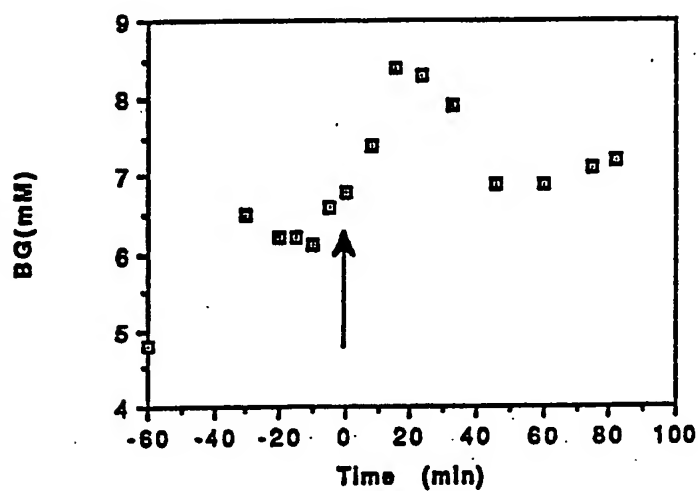


FIG 3B

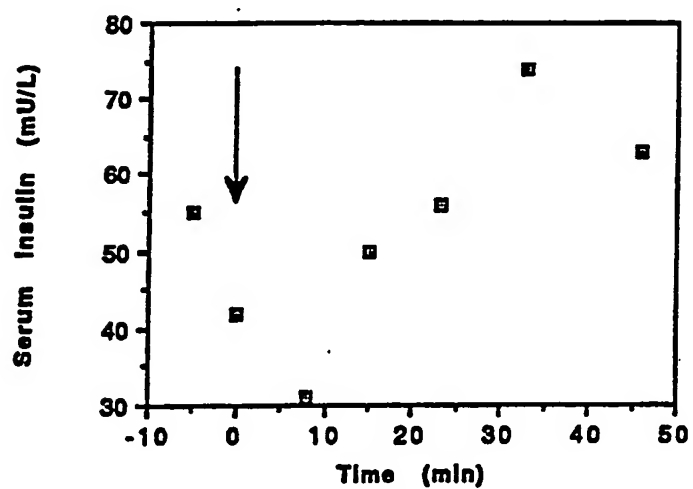
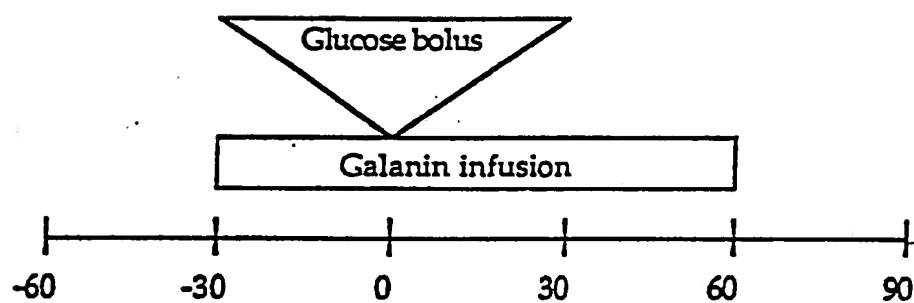


FIG 3C

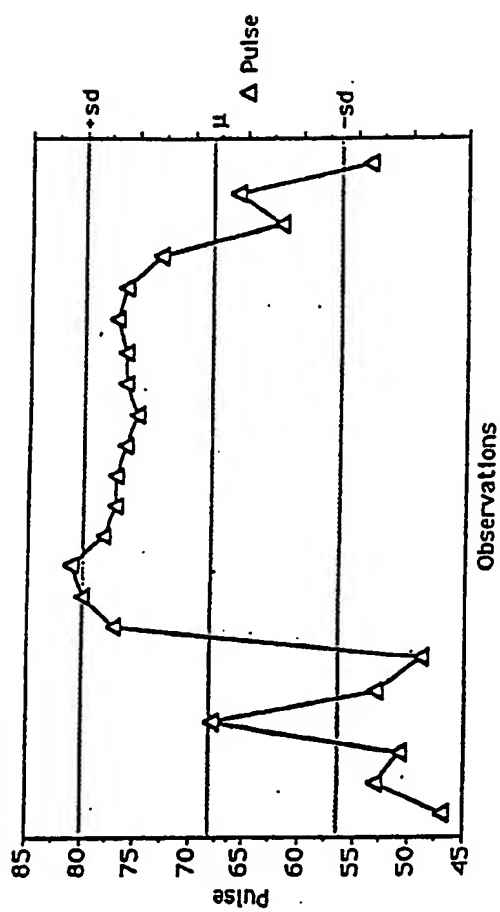
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Fig 4

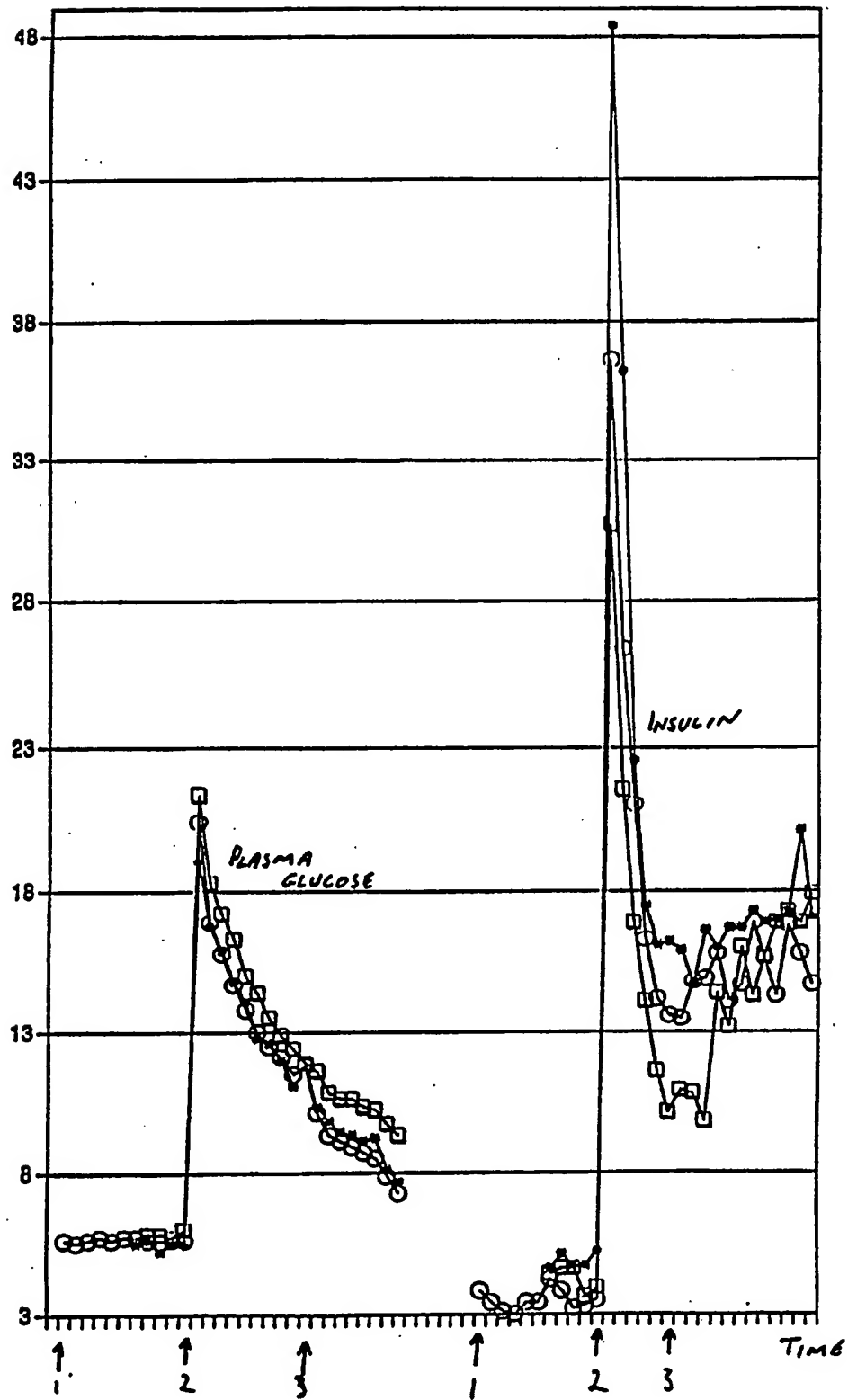


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Fig. 5



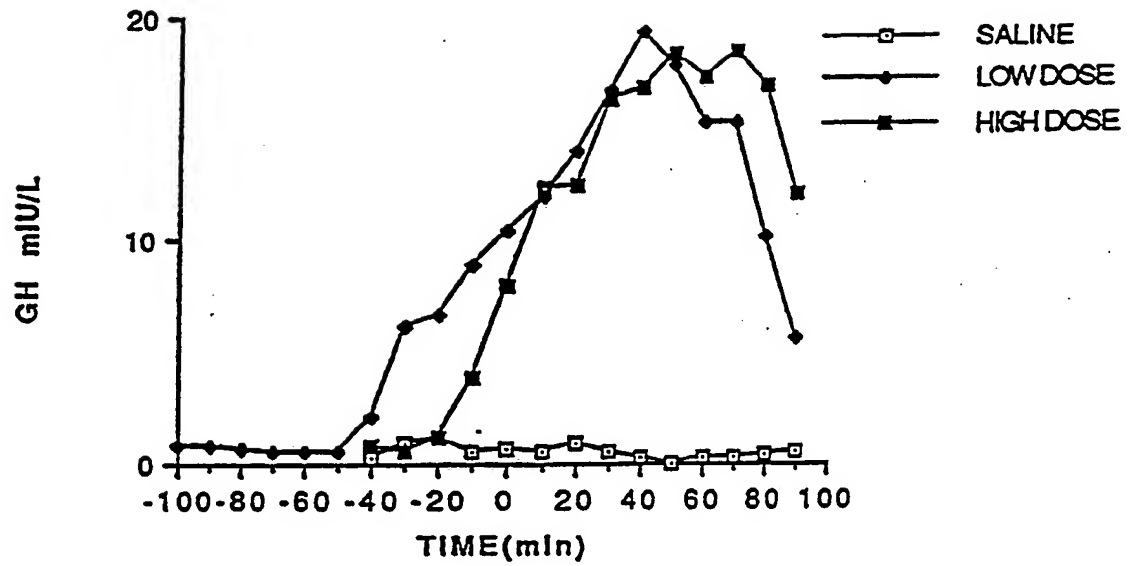
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FIG 6



SUBSTITUTE SHEET

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Fig 7



INTERNATIONAL SEARCH REPORT

| | | | | | | | | | | | | | | | | | | | | | | |
|--|--|--|--|--|-----|---|-----|--|-----|---|-----|---|-----|--|-----|--|-----|---|-----|--|--|--|
| I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ | | | | | | | | | | | | | | | | | | | | | | |
| According to International Patent classification (IPC) or to both National Classification and IPC Int. Cl. ⁸ C12N 15/12, C07K 7/10, A61K 37/02, A61K 37/43 | | | | | | | | | | | | | | | | | | | | | | |
| II. FIELDS SEARCHED | | | | | | | | | | | | | | | | | | | | | | |
| Minimum Documentation Searched ⁷ | | | | | | | | | | | | | | | | | | | | | | |
| Classification System | Classification Symbols | | | | | | | | | | | | | | | | | | | | | |
| IPC | Derwent Data base, WPAT: | | | | | | | | | | | | | | | | | | | | | |
| CAS | Keywords: human, galanin | | | | | | | | | | | | | | | | | | | | | |
| | Keywords: as above; | | | | | | | | | | | | | | | | | | | | | |
| Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸ | | | | | | | | | | | | | | | | | | | | | | |
| AU: IPC subclass C12 N 15/12; C07 K 7/10 Biotechnology Abstracts: keywords as above. STN data base: sequence search. | | | | | | | | | | | | | | | | | | | | | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ | | | | | | | | | | | | | | | | | | | | | | |
| Category ⁹ | Citation of Document, ¹¹ with indication, where appropriate of the relevant passages ¹² | Relevant to Claim No ¹³ | | | | | | | | | | | | | | | | | | | | |
| P,X | Endocrinology, vol. 129, No 3, 1991, pp 1682-1684. H.F. Evans and J. Shine "Human Galanin: Molecular cloning reveals a Unique Structure" | 1-9 | | | | | | | | | | | | | | | | | | | | |
| P,X | Neuroscience Letters, vol 136, 1992, pp 105-108. L.G. Ulman et al "Effects of human, rat and porcine galanins on cardiac vagal action and blood pressure in the anaesthetised cat" | 1-3, 5-9 | | | | | | | | | | | | | | | | | | | | |
| P,X | Proc. Natl. Acad. Sci. (USA), vol 88, Dec. 1991, pp 11435-11439. W.E. Schmidt et al "Isolation and primary structure of pituitary human galanin, a 30-residue nonamidated neuropeptide". | 1-3, 5-8 | | | | | | | | | | | | | | | | | | | | |
| <p>* Special categories of cited documents : ¹⁰</p> <table border="0"> <tr> <td>"A"</td> <td>Document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T"</td> <td>Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E"</td> <td>earlier document but published on or after the international filing date</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</td> </tr> <tr> <td>"L"</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>"&"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table> | | | "A" | Document defining the general state of the art which is not considered to be of particular relevance | "T" | Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | "E" | earlier document but published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step | "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | "O" | document referring to an oral disclosure, use, exhibition or other means | "&" | document member of the same patent family | "P" | document published prior to the international filing date but later than the priority date claimed | | |
| "A" | Document defining the general state of the art which is not considered to be of particular relevance | "T" | Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | | | | | | | | | | | | | | | | | | | |
| "E" | earlier document but published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step | | | | | | | | | | | | | | | | | | | |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | | | | | | | | | | | | | | | | | | | |
| "O" | document referring to an oral disclosure, use, exhibition or other means | "&" | document member of the same patent family | | | | | | | | | | | | | | | | | | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | | | | | | | | | | | | | | | | | | | | |
| IV. CERTIFICATION | | | | | | | | | | | | | | | | | | | | | | |
| Date of the Actual Completion of the International Search 25 June 1992 (25.06.92) | | Date of Mailing of this International Search Report 30 June 1992 (30.06.92) | | | | | | | | | | | | | | | | | | | | |
| International Searching Authority AUSTRALIAN PATENT OFFICE | | Signature of Authorized Officer K. AYERS <i>Kara Ayers</i> | | | | | | | | | | | | | | | | | | | | |

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

| | | |
|-----|--|----------|
| P,X | FEBS Lett., vol 283, No 2, June 1991, pp 189-194. M. Bersani et al "Human Galanin: primary structure and identification of 2 molecular forms" | 1-3, 5-8 |
| X | FEBS Lett., vol 234, No 2, July 1988, pp 400-406. A. Rokaeus and M. Carlquist "Nucleotide sequence analysis of cDNAs encoding a bovine galanin precursor protein in the adrenal medulla and chemical isolation of bovine gut galanin". | 1-8 |
| X | Proc. Natl. Acad. Sci. (USA), vol 85, February 1988, pp 1065-9. L.M. Kaplan et al "Tissue-specific expression of the rat galanin gene". | 1-8 |
| X | Proc. Natl. Acad. Sci. (USA), vol 83, September 1986, pp 6287-6291. A. Rokaeus and M.J. Brownstein "Construction of a porcine adrenal medullary cDNA library and nucleotide sequence analysis of two clones encoding a galanin precursor". | 1-8 |

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers, because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim numbers, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim numbers, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.